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ORIGINAL ARTICLE

# Fungal and bacterial co-infection in the superficial and deep sternal wound after open cardiac surgery

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## ABSTRACT

**Background and Objectives:** Sternum infection increases the time of the patients stay in the hospital and, as a result, increases the treatment costs. This study aimed to evaluate the fungal and bacterial co-infection in the superficial and deep sternal wounds after open cardiac surgery and its relationship with risk factors, as sternal infection increases the time of the patient's stay in the hospital and, as a result, increases the treatment costs.

**Materials and Methods:** Data were collected using a questionnaire and sampling with two swabs after open heart surgery and hospitalization from 21 March 2018 to 20 March 2019 and sent to the laboratory for diagnosis of microorganisms effective in wound infection. Susceptibility testing for fluconazole and specific antibiotics was performed by the disk diffusion method.

**Results:** Out of 210 patients studied, 2% of patients had deep sternal wound infections. The most common coinfection fungal and bacterial agents in sternal wounds were caused by *Staphylococcus aureus* with *Candida glabrata* 4% and *Escherichia coli* with *Candida albicans* 2%. *S. aureus* and *E. coli* showed the highest antibiotic susceptibility to the antibiotics ciprofloxacin, norfloxacin, meropenem, and imipenem. *Candida glabrata* and *Candida albicans* had the highest rate of resistance to fluconazole.

**Conclusion:** According to the results of this study, patients on the 7th day in the cardiac care unit (CCU) and the 28th day are at higher risk of getting confection of fungi with bacteria in the sternal wound. Therefore, timely and appropriate antibiotic therapy, including the use of appropriate antibiotics, can be an important step in the patient's recovery.

**Keywords:** Co-infection; Fungi; Bacteria; Risk factors

## INTRODUCTION

The infection of sternal wounds by sternotomy incision is an important and dangerous complication of thoracic and cardiac surgery (1- 3). Patients undergoing cardiac surgery are at significant risk of sternal wound infections that develops in the cutaneous and subcutaneous tissue (4, 5) along with complications such as osteomyelitis and mediastinitis (1). The prevalence of this infection can be attributed to a variety of causes, including factors related to the hospital environment, health settings, and length of hospital stay among patients (2, 6, 7).

The average time to onset of contamination is 7 to 28 days (8-10), which starts with indications such as arrhythmia, chest pain, and release (11), and other signs like fever, leukocytosis, positive blood culture, positive C-reactive protein (CRP) (10, 12, 13).

The most common pathogens causing sternal wound infections are *Staphylococcus, Streptococ-*

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*cus pyogenes*, Gram-negative bacilli, such as *E. coli*, *Proteus* and *Enterococcus*, *Mycoplasma*, and fungi also (14-16). Sternum wound infection is derived into superficial and/or deep forms. Various pathogenic microorganisms may lead to deep sternum wound infection (DSWI) via distinct mechanisms. DSWI has been shown in various studies to be associated with factors such as obesity and chronic obstructive pulmonary disease (COPD), often due to staphylococcal infection. Alternatively, this postoperative infection of the mediastinum is often caused by *Staphylococcus aureus*, while DSWI, along with the spread of bacteria from other sites, is often caused by Gram-negative bacteria (15, 17, 18).

Different species of *Candida* are serious causes of sternal wound infections. *Candida*-induced mediastinitis is a rare but important complication, with about 15% of the reported mortality rate that occurs within the first few days and weeks after surgery (16).

To date, few studies have documented the incidence of sternal wound infection (SWI) co-infection with fungi and bacteria or co-infecting species. One report described fungal and bacterial coinfections occurring at the same time in the SWI (1). At present, clinical treatment of fungal wound infections typically involves a combination of antifungal and antibacterial medications (2). Without a comprehensive understanding of fungal and bacterial infections, the experimental addition of antibacterial drugs may increase the risk of drug resistance. In addition, the possibility of co-infections raises concerns because it hampers the management of infections in sternal wounds, worsens the prognosis, and can increase mortality.

This study was conducted to investigate the co-infection of fungal and bacterial agents in superficial and deep sternal wound infection of open heart surgery patients and its relationship with related risk factors.

## MATERIALS AND METHODS

In this cross-sectional study, the frequency of fungal and bacterial co-infections in hospitalized patients in the Kordkuy Heart Center was assessed between 21 March 2018 to 20 March 2019. The present study enrolled 210 patients who underwent coronary artery bypass graft surgery was eligible for our present study. The inclusion criteria for our study included those who was a candidate for Coronary artery bypass graft (CABG). The exclusion criteria were as follows: partial sternotomy, patients with mechanical sternum dehiscence, and incomplete clinical data. Swabs provided information on the type of microorganism present in the wound and the susceptibility of the microorganism to specific antibiotics. Sampling was done from the sternum wound of coronary artery bypass surgery patients on the first day (ICU), the seventh day (CCU), and the 28$^{th}$ day after the surgery. Patients who were not present on all three sampling days were excluded from the study.

This study was performed on patients with CABG surgery at particular times postoperatively; the 1st day inside the Intensive Care Unit (ICU), the 7$^{th}$ day in Critical Care Unit (CCU), and the 28$^{th}$ day after surgery.

**Microbiological methods and antibiotic therapy.** To diagnose the causative agent of the infection, a sterile swab of the sternal wound discharge was prepared from patients who were hospitalized for 24 hours in ICU, seven days in CCU, and 28 days after CABG surgery. Swab samples were cultured on Blood Agar (BA), and MacCankey Agar (Mac A) media with an incubation time of 24-72 hours, and culture growth condition was assessed. Then, for isolation, a loopful of the identified colonies was streaked over a new blood agar plate and incubated aerobically at 37°C for 24 h. Next, from culture-positive plates, typical colonies were subjected to Gram's staining to study staining reactions and cellular morphology under a light microscope. To detect fungal pathogens samples were obtained and cultured on Sabouraud Dextrose Agar (SDA) with chloramphenicol. All fungi specimens were analyzed for phenotypic detection by microscopy direct slide with KOH (10%). Wet mount direct smears were examined under the microscope. When no growth was observed in the culture media, the plates were stored for up to 72 hours. For identification, conventional biochemical tests such as nitrate reduction, oxidase, Triple Sugar Iron Agar (TSI), Simmons Citrate Agar, Sulfide-Indole-Motility (SIM) media, urease, phenylalanine deaminase, lysine decarboxylase, catalase test, coagulase test, mannitol fermentation, and DNase, OF Test, the culture medium of methyl red Voges-Proskaue (VP) was performed.

Co-infection was defined by clinical signs and/or symptoms of bacterial or fungal infection alongside with detection of a pathogen by a diagnostic test.

**Detection of fungal species (Phenotypic and Genotypic).** For fungal species identification, sampling was performed by wet swabs from patients who were hospitalized for 24 hours in ICU, seven days in CCU, and 28 days after CABG surgery. Samples were cultured in Sabourod Dextrose Agar (Merck Co., Germany) medium containing chloramphenicol. The colonies appeared a few days after incubation (3 to 4 days). Also, for the primary detection of *Candida* species, CHROMagar *Candida* medium as a new differential isolation medium was used. After confirming the macroscopic morphology and then staining with Lactophenol Cotton Blue (LCB), the suspected samples were cultured on Sabouraoud's dextrose agar and incubated at 32ºC for 48 h. One colony on each plate was selected and sub-cultured for genotyping. Genomic DNA was extracted through the method of glass bead disruption. PCR amplification of ITS1-5.8S-ITS2 rDNA regions was attained using the ITS1 (forward, 5′-TCC GTA GGT GAA CCT GCG G-3′ and ITS4 (reverse, 5′-TCC TCC GCT TAT TGA TAT GC-3′) primer pairs. To amplify ITS domains, PCR amplification was achieved in a final volume of 50 µl. Each reaction consists of 2 µl template DNA, 0.5 µl of each primer at 25 µM, 1.25 µl of dNTP at 5 mM, 0.5U *Taq* DNA polymerase (Genet bio), and 5 µl 10× PCR buffer. The amplification parameters consist of 35 cycles of denaturation at 94°C for 1 min, primer annealing at 56°C for 1 min, and extension at 72°C for 1 min. In the first cycle, the denaturation step was 94°C for 5 min and in the final cycle, the final extension step was 72°C for 7 min. Amplified products were visualized by 1% agarose gel electrophoresis in TBE buffer (20 mmol/l EDTA, 10 mmol Tris boric pH 8). The gel was stained with ethidium bromide (0.5 µg/ml) and photographed by ultraviolet photography.

Each PCR product was digested with FastDigest MspI. (Thermo Fisher Scientific, USA). 10 µL PCR product, 17 µL nuclease-free water, 2 µL 10×buffer, and 1 µL enzyme were incubated at 37°C for 30 min. PCR and RFLP products were separated on a 1.5% agarose gel in TBE buffer for 45 min at 100 V and visualized by staining with ethidium bromide. Previously reported PCR-RFLP profiles were used to identify yeast species (10, 11).

**Restriction fragment length polymorphism analysis.** Digestion of amplified products was performed using restriction enzyme MspI. The digestion of the ITS region of *Candida* spp. by the MspI enzyme created two bands for *C. albicans*, *C. glabrata*, *C. tropicalis*, and *C. krusei*. For *C. parapsilosis*, the size of the PCR and digestion product was similar. The restriction patterns of each *Candida* spp. were perfectly specific. The sizes of amplified DNA fragments were identified by comparison with molecular size marker DNA (100-bp DNA ladder) (15).

**Detection of antimicrobial resistance.** To detect antimicrobial resistance, the disk diffusion method of the Kirby-Bauer was used (16). Norfloxacin, nitrofurantoin, sulfamethoxazole/ trimethoprim, clindamycin, ceftriaxone, and fluconazole were used. After this, the zone around the disks was examined for growth inhibition, and the sensitivity and resistance of bacteria to antibiotics were determined according to the CLSI-M100 (16).

**Statistical analysis.** The statistical analyses were performed by the software SPSS version 16. Descriptive statistics were presented as mean ± SD and proportions as appropriate. We used the t-test or Chi-square test to compare differences. The results with a P-value $< 0.05$ were considered statistically significant.

**Ethics approval and consent to participate.** The study was conducted after it was ethically reviewed and approved by the Ethical Review Board of the Golestan University of Medical Sciences Ethics Committee (Approval Code: IR. Gums. rec. 1397.095).

Patient demographic information was collected by age, sex, and clinical information of inpatients referred to Amiralmomenin Hospital of Kordkuy. Written consent were obtained from patients.

## RESULTS

**Epidemiologic characteristics.** From 21 March 2018 to 20 March 2019, 210 patients developed sternal wound infections following heart surgery. 30% of patients reported a sternal infection. Most of the micro-organisms isolated from SWI have been reported in men.

**Microbiological characteristics.** On the first and 7$^{th}$ days, *Staphylococcus aureus* was found with a frequency of 50% and 33%, respectively. On the 28$^{th}$ day, a greater variety of microorganisms was observed. So the prevalence of *Staphylococcus aureus* was 22%.

According to the data on the first day, there is a statistically significant relationship (P-value<0.05). But, on the 7th and 28th day, there was no statistically significant relationship (P-value= 0.58, P-value= 0.55 respectively) between sex and types, obesity, and underlying disease of microorganisms isolated (Table 1).

According to the fungal and bacterial isolates from sternal ulcers postoperative, it was found that Gram-positive bacteria had a higher frequency and diversity than Gram-Negative bacteria. In addition, Gram-positive bacteria played the greatest role in the infection of the sternal wound infection on the 1st in ICU, 7th in CCU, and 28th days after surgery. On the 28th day after surgery in discharged, a much greater variety of isolates was seen compared to the both 1st and 7th days postoperative was reported. Moreover, the highest percent of Gram-positive bacteria were related to *Staphylococcus aureus* (30%) on the 1st day. On the 28th day, the majority of isolated Gram-negative SWI bacteria were related to *Escherichia coli*. Examination and isolation of *Candida* species showed a high percentage of *Candida glabrata* (10%) and a lower percentage of *Candida tropicalis* (2%). Out of 10% of *Candida glabrata* isolates isolated from wounds of open heart surgery patients, only 2% caused infection in the sternum. Out of 2% of *Candida tropicalis* isolated from open heart surgery wounds, 1.7% caused infection in sternum wounds (Table 2).

Out of 210 patients, 67% were male, and the remaining were female. The mean age of the patients was 44.76 years (Table 1). Based on the color code on CHROMagar *Candida*, 75 of the 78 *Candida* isolates were identified 29 as *C. tropicalis*, 14 as *C. albicans*, 16 as *C. glabrata*, 10 as *C. parapsilosis*, 9 as *C. krusei*, and 3 unidentified (Fig. 1). By using the PCR-RFLP technique, all of the isolates were further genotypically identified. Using universal primers, the ITS1 and ITS4 successfully amplified the ITS region of 78 clinical isolates from the examined *Candida* spp. and it produced a distinctive PCR product with a size of roughly 510-881 bp. According to Fig. 1, all *Candida* isolates identified as *C. albicans* based on color (light green)

**Table 2.** Prevalence of fungal and bacterial infections in 1 day (ICU), 7 days (CCU), and 28 days after open heart surgery patients

| Fungal and bacterial infections | Number (%) |
|---|---|
| Fungal and bacterial infections | 63/210 (30%) |
| *Staphylococcus areus* | 22 (12%) |
| Strep group A | 2 (1.1 %) |
| *E. coli* | 12 (7.3%) |
| *Enterobacter* | 5 (2.8%) |
| *Pesudomunas* | 8 (4.5%) |
| *Proteus* | 2 (1.7%) |
| *Citrobacter* | 2 (1.1%) |
| *Candida kruzei* | 2 (1.1%) |
| *Candida glabrata* | 4 (2%) |
| *Candida albicans* | 3 (1.7%) |
| *Candida parapsilosis* | 2 (1.1%) |
| *Candida tropicalis* | 3 (1.7%) |

**Fig. 1.** Morphology *Candida* species in *Candida* chrome agar medium

**Table 1.** Demographic and clinical characteristics of the included patients

| Patient/disease characteristics | Total patients (n = 210) | Co-infected group (n = 22) | The non-co-infected group (n = 188) | P value |
|---|---|---|---|---|
| Mean age (year) | 55.22 ± 9.91 | 4.03 ± 50 | 47 ± 2.04 | 0. 6 |
| Sex | | | | |
| Male | 141 | 4 | 149 | 0.76 |
| Female | 69 | 2 | 65 | |
| Risk factors | | | | |
| Hypertension | 169 | 1 | 157 | >0.05 |
| Diabetes | 109 | 3 | 90 | 0.03 |
| Chronic lymphocytic leukemia | 7 | 1 | 5 | 0.4 |
| Obesity | 145 | 2 | 127 | 0.001 |
| underlying disease | 87 | 1 | 99 | 0.01 |

by CHROMagar *Candida* were in agreement with PCR-RFLP while, the identification of the non-*albicans Candida* (*C. krusei*, *C. glabrata*, *C. parapsilosis*, and *C. tropicalis*) by color on CHROMagar Candida showed a discrepancy with PCR–RFLP. The minimum time required for the identification of 78 samples of *Candida* species in our study included 24 hours for Chrom agar *Candida* and PCR- RFLP (Fig. 2).

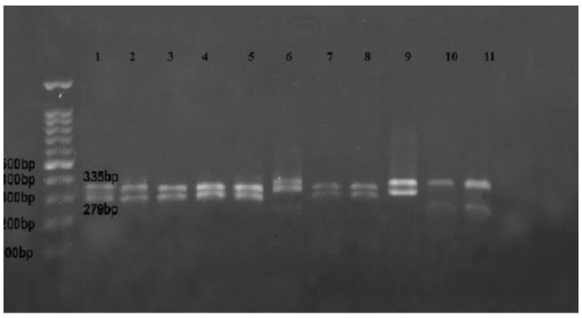


**Fig. 2.** Patterns of PCR products of *Candida* isolates after digestion by the restriction enzyme Msp1.
Lanes 1-5, 7, 8 represent *C. albicans*; and 6, *C. kruzei;* 9, *C. parapsilosis*; 10, 11, *C. tropicalis* Lane M is a 100 bp ladder molecular size marker.

The chromogenic *Candida* differential media results are shown in Fig. 1. The results of chromogenic agar only showed a mix of yeasts; however, they could not identify the species, whereas the current PCR-RFLP approach successfully identified the species in mixed cultures.

In this study, out of 210 CABG samples, finally, 78 samples had *Candida*-positive colonies. The results of species identification of candida colonies by PCR-RFLP method showed five species of *Candida* including *Candida albicans*, *Candida glabrata*, *Candida krusei*, *Candida tropicalis*, and *Candida parapsilosis* (Figs. 1 and 2).

**Fungal and bacterial co-infection in SWI.** Among 178 culture-positive patients, 17 (8%) had a polymicrobial infection of the sternal wound. The risk of polymicrobial SWI was increased with obesity (p = 0.005), and smoking, alcoholism. Age had no significant effect on the occurrence of polymicrobial infection.

The most common microbes isolated from sternal wound were *Staphylococcus aureus* (8) mixed with *Candia glabrata* , *E. coli* mixed with *Candia albicans* (4), *Enterobacter* mixed with *Staphylococcus aureus* (3), *Pseudomonas aeruginosa* mixed with *Staphylococcus aureus* (2) in 4 patient Deep SWI and 13 of superficial SWI. The maximum age range from which microorganisms were separated in each of the three days was 47-80 years, which is described in Table 1 and Figs. 3 and 4.

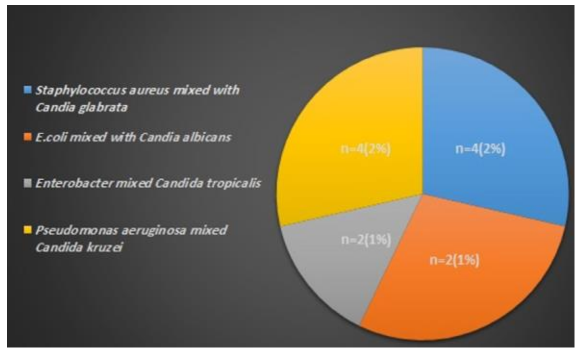


**Fig. 3.** Prevalence of co-infection fungal and bacterial in sternum wound

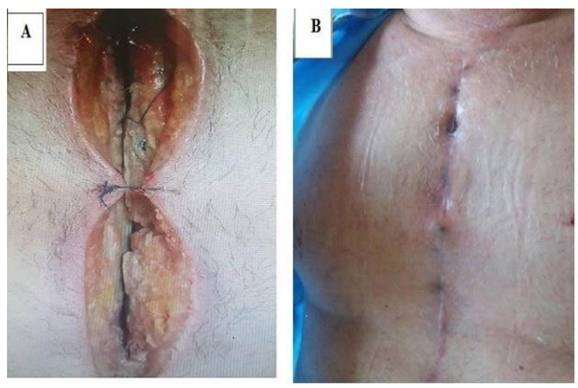


**Fig. 4.** Deep superficial infections after open heart surgery.

**Antibiotic susceptibility and resistance of bacterial isolates.** Patterns of susceptibility and resistance of Gram-negative and positive bacteria isolated from wound infection against 10 antimicrobial agents were investigated (Table 3). Among 210 positive cultures patients, 15 patients had positive blood cultures, and out of bacteria involved in SWI, *Staphylococcus aureus*, *E. coli*, and *Enterobacter* were more sensitive to the antibiotics such as ciprofloxacin, norfloxacin, and sulfamethoxazole. Among the isolated bacteria, *Streptococcus* group A and *Pseudomonas aeruginosa* were resistant to all used antibiotics, respectively (Table 3).

**Antifungal susceptibility and resistance of yeasts isolates.** Of all the yeasts isolated in the 1st ICU, 7th CCU, and on the 28th days after heart surgery 33, 21, and 31 cases were positively cultured, respectively. Drug resistance and susceptibility to the antifungal

**Table 3.** A pattern of antibiotic resistance and susceptibility of bacteria isolated from patients with SWI

| **Antibiotics** | ***E. coli*** n =12 (%7.3) | | | ***S. aureus*** n =22 (12%) | | | ***Enterobacter*** n =3 (1.1%) | | | ***P. aeruginosa*** n =8 (4.5%) | | | ***Streptococcus* group A** n =2 (1%) | | | ***Proteus*** n = 2 (1.7%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pattern of antibiotic | **I** | **S** | **R** | **I** | **S** | **R** | **I** | **S** | **R** | **I** | **S** | **R** | **I** | **S** | **R** | **I** | **S** | **R** |
| Ciprofloxacin | 3 | 6 | 3 | 5 | 17 | - | - | 3 | - | - | 2 | 6 | - | - | 2 | | - | - |
| | 25% | 50% | 25% | 16.6% | 58.4% | | | 100% | | | 25% | 75% | | | 100% | | | |
| Ceftazidime | 3 | 3 | 6 | 4 | 6 | 12 | 2 | - | 1 | - | | 8 | - | - | 2 | 2 | - | - |
| | 25% | 25% | 50% | 19% | 24% | 57% | 75% | | 25% | | | 100% | | | 100% | 100% | | |
| Gentamicin | 3 | 6 | 3 | 4 | 18 | - | 1 | 2 | - | - | 2 | 6 | - | - | 2 | 2 | - | - |
| | 25% | 50% | 25% | 19% | 81% | | 25% | 75% | | | 25% | 75% | | | 100% | 100% | | |
| Imipenem | - | 8 | 4 | 4 | 18 | - | | 3 | - | - | 2 | 6 | - | - | 2 | 2 | - | - |
| | | 67% | 33% | 19% | 81% | | | 100% | | | 25% | 75% | | | 100% | 100% | | |
| Meropenem | 6 | - | 6 | 12 | 12 | - | 1 | 2 | - | - | 1 | 6 | - | - | 2 | - | 2 | - |
| | 50% | | 50% | 50% | 50% | | 25% | 75% | | | 25% | 75% | | | 100% | | 100% | |
| Norfloxacin | - | 8 | 4 | 5 | 17 | - | - | 3 | - | - | 1 | 6 | - | - | - | 2 | - | - |
| | | 64% | 33% | 22% | 78% | | | 100% | | | 25% | 75% | | | | 100% | | |
| Nitrofurantoin | 3 | 6 | 3 | 5 | 17 | - | - | 3 | - | - | 4 | 4 | | 1 | - | 2 | - | - |
| | 25% | 50% | 25% | 22% | 78% | | | 100% | | | 50% | 50% | | 50% | | 100% | | |
| Sulfamethoxazole / | - | 9 | 3 | 4 | 18 | - | - | 3 | - | - | 4 | 4 | - | - | - | 2 | - | - |
| Trimethoprim | | 75% | 25% | 18% | 82% | | | 100% | | | 50% | 50% | | | | 100% | | |
| Clindamycin | 6 | - | 6 | 1 | 21 | - | 1 | 3 | - | - | 2 | 6 | - | - | 2 | - | - | - |
| | 50% | | 50% | 5% | 95% | | 25% | 100% | | | 25% | 75% | | | 100% | | | |
| Ceftriaxone | 2 | 6 | 4 | 4 | 4 | 14 | 1 | 2 | - | - | - | 4 | 4 | - | 2 | - | - | 2 |
| | 15% | 50% | 25% | 18% | 18% | 64% | 25% | 75% | | | | 50% | 50% | | 100% | | | 100% |

fluconazole were evaluated (Table 4). Examination of the results showed that in all three sampling sessions, the highest resistance to the relevant fluconazole was observed in *Candida glabrata* (100%). On the other hand, in the 1st and 7th days, the highest resistance in the *Candida glabrata*, and *Candida albicans* to the antifungal fluconazole (100%, 50%) was reported. On the 28th, in addition to *Candida glabrata, Candida krusei* and, *Candida parapsilosis* showed the highest resistance to fluconazole (100%). All *Candida* species were susceptible to amphotericin B (Table 4).

Fungal and bacterial co-infection was observed in about 17 patients (8% out of 210) who underwent coronary artery bypass surgery. Among these 17 patients, 13 (6%) cases of superficial and 4 (2%) cases of deep sternal infection were seen. Patients with sternal infection were mostly men (52.6%). They had high weight, and most of them had 45-56 ages and had diabetes.

Wound debridement and a closed drainage system were placed in most patients with sternum superficial and deep wounds (22 %) up to 48 hours after diagnosis of infection. Two patients with deep sternal infection underwent tracheostomy due to prolonged ventilator use. Patients whose diagnosis of sternum infection was delayed were repaired after extensive debridement of the skin, subcutaneous tissue, and removal of a large part of the sternum.

## DISCUSSION

This study was conducted to investigate the co-infection of fungal and bacterial agents in superficial and deep sternal wound infection of open heart surgery patients and its relationship with related risk factors. We found 4 patients had DSWI; the incidence was 2%. This goes with Cotongi et al. (1) as the incidence of deep sternotomy wound infection was 4.6%, Yusuf et al. (11) reported the incidence as 2.28%, but in the study of Hosseinrezaei (12) it was 1.54%.

Our results showed that among 30% of CABG patients with sternal wound infections, 8% had fungal and bacterial co-infections. Clinical manifestations of fungal and bacterial infections in CABG patients were much more serious than fungal infections alone in these patients.

**Table 4.** Pattern of antibiotic resistance and susceptibility of fungal isolated from patients with SWI.

| Antifungal agents | *Candida glabrata* (n=4) | | | *Candida albicans* (n=3) | | | *Candida parapsilosis* (n=2) | | | *Candida kruzei* (n=2) | | | *Candida tropicalis* (n=3) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **R** | **I** | **S** | **R** | **I** | **S** | **R** | **I** | **S** | **R** | **I** | **S** | **R** | **I** | **S** |
| Fluconazole | 4 | - | | 2 | 1 | - | - | 2 | - | 2 | - | - | 1 | 2 | - |
| | (100%) | | | (66%) | (34%) | | - | (100%) | | (100%) | | | (34%) | (66%) | |
| Amphotricine B | - | 3 | 1 | - | 3 | - | | - | 2 | - | - | 2 | - | - | 3 |
| | | (66%) | (34%) | | (100%) | | | | (100%) | | | (100%) | | | (100%) |

Fungal infections commonly occur in immunocompromised individuals. However, in our study, a large proportion (59%) of patients with sternum wound fungal infections had a normal immune function. An increasing number of reports show that immunocompetent patients suffer from fungal infections, probably due to environmental exposures, and genetic factors.

Obesity, older age, insulin-dependent diabetes, and other preoperative comorbidities have also previously been identified as risk factors for deep sternal infections (2, 19). Our data also showed that diabetes was a major risk factor for CABG patients (52%). In this regard, this disease was associated with the frequency of isolation of microorganisms and hospital infection (chi-square test, P value: 0.06). In the study of Bartoletti et al. (2019), they reported that the most common risk factor for diabetes was obesity in 89 of cases and about 69% of cases (20).

Older age has also been identified previously as a risk factor for deep sternal infections (20-22). In a study conducted by Parissis et al. (2011) on 167 patients who underwent heart surgery and had a sternal wound infection, the men's gender (n=89, 53.3%) and the age over 60 years (n=104, 62.3%), 109 (52%) obesity, and 87 (42%) underlying disease was the dominant individuals in this study (23). In this study, the men's gender (n=141, 67%) and the age over 55 years (n=104, 49%), 145 (69%) obesity, and 87 (41%) underlying disease were dominant.

According to the results of various studies, it should be noted that long-term hospitalization of CBAG patients with underlying diseases can increase the risk of hospital infections, especially fungal infections because each of these diseases can act as a risk factor and increase the risk of hospital-acquired infections (23-25). In our study, the majority of sternum wound fungal infections were seen in 52% of diabetic and 11% of hypertension patients in 28 day superficial sternum wound infection, which related to *Candida glabrata* with *S. aureus* and *E. coli* with *Candida albicans.*

The results of the study by Hariri et al. (2023), which was performed on 11 patients who underwent heart surgery, showed that 91% of the patients were male and their mean age was 65-72 years (26). Isolates such as *Candida albicans* (6 cases), *Candida albicans* with *Staphylococcus coagulase* negative (3 cases), and *Candida albicans* with *Enterococcus faecalis* (1 case) were reported in patients with SWI (26). In our study, the highest prevalence of fungal and bacterial co-infections was related to *Candida glabrata* and *Staphylococcus aureus* 4% (8 cases), and the lowest percentage of prevalence was related to *Candida albicans* and *E. coli* 2% (4 cases) in CABG patients.

In a recent study, deep sternal infection occurred in 2% of patients who underwent CABG surgery and caused a significant increase in the mortality of patients. More deaths were related to patients with deep sternal infections who underwent the second surgery 48 hours after the infection diagnosis. It is noteworthy that most patients with superficial sternum infection underwent surgery within 48 hours after the infection was diagnosed.

At our center, a routine antibiotic regimen includes intravenous cefazolin as empiric therapy for sternal purulent ulcer prophylaxis regardless of whether it is considered "superficial" or "deep" on initial evaluation. The duration of empiric therapy is usually longer because antibiotics are prescribed only after the results of deep sternal swabs are available.

Several studies have evaluated the pathogen spectrum in SWI patients after heart operations. Chaudhuri et al. in their study performed between 2017 and 2019 (27) showed that *Staphylococcus aureus* was the commonest organism cultured from deep sternal wounds (39%). Half of the cultured organisms were resistant to ceftazidime, and ceftriaxone, while Gram-negative organisms were cultured in less than a quarter of patients 27 (15%). *E. coli* was the most common Gram-negative organism isolated across samples (7.3%).

In our research, the most common bacterial isolates isolated from the sternal wounds were *S. aureus* (12%), *Streptococcus* Group A (1.1%), *E. coli* (7.3%), *Pseudomonas aeruginosa* (4.5%) and *Candida* species (7.5%). These results were consistent with the results of the study of Hosseinrezaei et al. (12).

In the study, Guan et al. and Gadepalli et al. the resistance level of *E. coli* to ciprofloxacin and levofloxacin was high (more than 60%) and only 7.3% of isolates were sensitive to meropenem, amikacin, and nitrofurantoin. We found less than 5% resistance of *Enterobacter* species to imipenem. Gram-negative bacteria are highly sensitive to amikacin and meropenem (28, 29).

In a study conducted by Heydarpour et al. out of 600 patients with open heart surgery during the four years of the study, 135 patients, including males (40.7%) and females (59.3%) with a mean age of 8.06 ± 3.86 months, were enrolled in the study. The prevalence of infection was 11.96%. The most commonly isolated bacteria were *E. coli*, *Klebsiella* spp, as Gram-negative ones, and *S. aureus* as Gram-positive types. *E. coli* was further found mainly susceptible to imipenem (23%), *Klebsiella* spp. to gentamicin (38.5%), and *S. aureus* to co-trimoxazole (54.2%) (30). Contrary to the above results, in our study, including males (67%) and females (23%) with a mean age of 55.22 ± 9.91, the predominant microorganisms to produce co-infection isolated were *Staphylococcus aureus* with *Candida glabrata* (4%) and *E. coli* with *Candida albicans* (2%). *Staphylococcus aureus* was mainly susceptible to ciprofloxacin (58.4%), and *E. coli* to imipenem (67%).

*Candida parapsilosis* was susceptible to fluconazole but *Candida glabrata* and *Candida albicans* were found mainly resistance to this drug. All *Candida* species were sensitive to amphotericin B.

Fluconazole is the most common antifungal agent used for prophylaxis and treatment of *Candida* infections. However, several *Candida* species were resistant to it. In the present study, 8 cases were infected with *C. glabrata* of which 50% were resistant to fluconazole but sensitive to amphotericin B. Therefore, in this study, amphotericin B was used to treat isolates resistant to fluconazole.

The results of our studies showed that non-albicans species play an effective role in the occurrence of candidal mediastinitis infections and resistance to fluconazole.

## CONCLUSION

The overall incidence rate of SWI was 30%. The most common fungal agents isolated on the 7th and the 28th day were *Candida glabrata* and *Candida albicans,* which had the highest resistance rate to fluconazole. The most common bacterial agents identified were *Staphylococcus aureus,* and *Enterococcus*, which had a high rate of resistance to ciprofloxacin, norfloxacin, and sulfamethoxazole. The highest occurrence of sternal wound infection (SWI) was shown with *Staphylococcus aureus* and *Candida glabrata*.

In the period of the study, the incidence of DSWI was 2%. Deep sternum infection causes a significant increase in the death and morbidity of patients. Therefore, clinicians by accurately identifying and controlling risk factors such as length of hospitalization, obesity, and underlying diseases in open heart surgery patients, can prevent the occurrence of sternal.


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